electrostatically stabilized gel. The rigidity of the gel, indicative of the interaction strength and range, can be quantified by measurement of the elastic modulus G′. We obtain G′ by optically trapping and oscillating a polystyrene particle in the gel and measuring the amplitude and phase of its displacement. From the screened Debye-Hückel potential, G′ is related to an *effective* particle charge Z*, as described by Alexander et al. (1984). Thus we determine Z* as a function of Z_0. The particle charge Z in the presence of interactions can be calculated from G′ by numerical integration of the Poisson-Boltzmann equation (Alexander et al., 1984). In deionized solutions, because there is 1 dissociated H^+ for each particle charge, higher Z* is accompanied by more H^+ and more screening. At low Z*, the interaction is dominated by the particle charge, and G′ increases as Z^{*3}. At high Z*, the interaction is dominated by H^+ screening, and G′ decreases as $\exp(-Z^{*1/2})$. We demonstrate this effect experimentally and determine Z* at G'_{max} for several liposome concentrations. A G′ maximum can still exist in the presence of low ionic-strength salt when $[H^+]$ is comparable to the concentration of salt counterions. Our results show that the elastic moduli of electrostatically stabilized colloidal suspensions in low ionic-strength media are maximal at intermediate values of the particle charge.

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Quantum and All-Atom Molecular Dynamics Simulations of Protonation and Divalent Ion Binding to Phosphatidylinositol 4,5-Bisphosphate (PIP2)
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Phosphatidylinositol 4,5-bisphosphate (PIP2) is a minor component of the inner plasma membrane that is capable of binding to hundreds of intracellular proteins. It carries a large negative charge, has a big lateral surface area, and can form clusters under certain ionic conditions in vitro. We have completed an analysis of the structure of PIP2 at the quantum level and the propensity for PIP2 to bind physiologically relevant divalent cations. We performed a geometry optimization at the Hartree-Fock 6-31G(d) level of theory in vacuum and with a polarized continuum dielectric to determine the conformation of the phospholipid headgroup in the presence of water and its partial charge distribution. The angle between the headgroup and acyl chains is approximately 89 degrees, indicating that the inositol ring may lie flat along the surface of the inner plasma membrane. Next, we employed hybrid quantum mechanics/molecular mechanics (QM/MM) simulations to investigate the protonation state of PIP2 and its interactions with divalent cations such as magnesium or calcium. We test the hypothesis that binding of magnesium to PIP2 is mediated by a water molecule that is absent when calcium binds. We observe that binding of calcium is able to deprotonate PIP2 at one phosphate group, which causes the molecule to decrease in size, and this does not occur when magnesium binds. These results may explain the ability of calcium to induce the formation of PIP2 clusters and phase separation from other phospholipids.

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Membrane Charging and Interfacial Hydration
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Due to thermal motion and molecular polarizability, physical interactions at the membrane-water interface have a pronounced dynamic character. In particular, the interplay between molecular disorder (entropy) and molecular interactions can have unexpected consequences primarily with regard to membrane electrostatics [1,2]. We show experimentally that significant charging occurs for lipid membranes in the presence of highly polarizable solutes such as anions and zwitterionic pH buffers. To complicate matters, this charging process takes place while there is a net deficit of solutes in the immediate vicinity of membranes. The important consequence is that electrostatic forces between macromolecular surfaces are then less screened and can act over long distances. We quantify both membrane electrostatics and solute deficit by using a number of experimental methods including small-angle x-ray scattering, buoyancy measurements, and molecular drift in electric fields. It is also shown that there are solute mixtures in which electrostatic cancellation occurs. These aspects of membrane electrostatics are relevant to studies of membrane fusion and of protein-lipid membrane interactions.
[1] Petrache *et al.* J. Am. Chem. Soc. 2005, [2] Koerner *et al*. Biophys. J. 2011.

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Intramolecular Hydrogen Bonds in Cardiolipin
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Cardiolipin was first isolated in 1941, and has since been implicated in several critical physiological roles. The distribution of cardiolipin species in tissues across prokaryotes and eukaryotes, and its concentration in membranes across which proton gradients drive energy production, is well characterized. The various functions attributed to cardiolipin include the structural stabilization of oxidative phosphorylation complexes in the inner mitochondrial membrane, where it constitutes about 25% of lipid. Its acid-base titration profile suggests a variable head group pKa in the physiological range, which leads to the hypothesis that cardiolipin also serves as a proton reservoir for energy-related protein complexes in the mitochondrial inter membrane space, thylakoid lumen, and at the outer leaflet of bacterial membranes. The importance of cardiolipin in these ascribed roles is further underscored by its involvement in diseases such as Barth's syndrome, where mitochondrial dysfunction is a major factor.
In contrast to this physiological importance, Cardiolipin remains one of the least well characterised lipids from a biophysical perspective. In particular, there is still no consensus on whether it is a hydrogen bond between a protonated phosphate and the free 2-hydroxyl of the bridging glycerol of cardiolipin that explains its anomalous pKa. Furthermore, static 31P solid-state NMR characterisations of the head group have been difficult to interpret. We use our new Boltzmann-statistics maximum entropy analysis to globally analyse slow magic angle spinning spectra, and explain the uncharacteristic 31P static NMR spectra. Together with differential scanning calorimetry analysis, intriguing evidence emerges for the hydrogen bond hypothesis. We further pursue the hypothesis with additional solid-state NMR 31P{1H} heteronuclear correlations (HETCOR) and rotational-echo double-resonance (REDOR) experiments.

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Water Pockets between Acyl Chains and its Relation to Peptide Insertion in Lipid Membranes
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Presence of water in the membrane structure has been invoked to interpret the insertion of highly hydrophilic aminoacid moieties in pools between acyl chains, described as water pockets. Analysis of the band corresponding to the frequency of vibrational symmetric stretching mode of methylenes and the bands of water below and above the phase transition of different lipids by Fourier transform infrared spectroscopy give strong support to the formation of confined water pockets in between the lipid acyl chains. We present a rational description of the water pockets creation and the influence of the adjacent wall formed at the phase transition by analyzing the changes of FTIR-ATR spectra in the regions corresponding to the CH2 and water bands shifts with temperature. The presence of water in these packing defects gives support to the creation of regions with free energy excess due to reinforcement of the water structure. This high energy defects explains the insertion of peptides and aminoacid residues and translocation of peptides through the biomembrane.

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Volumetric Stability of Lipid Bilayers
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The gel phase of DPPC has been the best characterized lipid bilayer. It has therefore been alarming that recent publications have reported a gradual decrease in lipid molecular volume of DPPC multilamellar vesicle dispersions in the gel phase upon repeated thermal cycling between 10°C and 50°C using a commercial densimeter. The considerable size of this decrease would have significant implications for the physical chemistry of biomembranes. We have confirmed this phenomenon with the same densimeter model. By contrast, neutral buoyancy measurements performed with similar thermal cycling show no gradual change in lipid volume in the gel phase at 20°C. Remixing the lipid in the densimeter shows that the apparent volume decrease is an artifact. We conclude that volumes obtained by neutral buoyancy measurements remain accurate and that gel phase DPPC bilayers exist in a volumetrically stable state.
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Interactions of a Monofluorinated Phospholipid with Saturated Phosphatidylcholines of Different Chain Lengths
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This study uses differential scanning calorimetry (DSC) and fluorescence spectroscopy to study the thermodynamic and kinetic effects of the monofluorinated